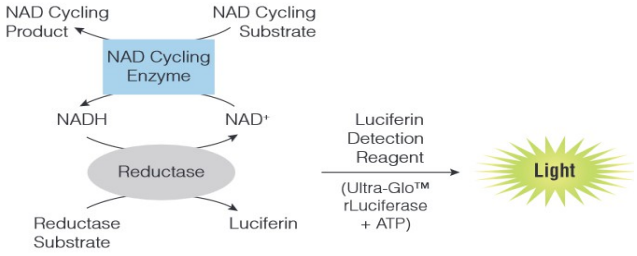
Promega NAD/NADH-Glo™ Assay

- Basic principle of the enzymatically detection assay:



NAD/NADH-Glo™ Assay

- Protocol:
  - The 1st step, before the assay can be performed is the lysis of the cells.
  - 1 ml of bacteria culture (OD_{600}= 0,375 ≈ 3 • 10^3 cells)get pelleted by centrifugation
  - Remove supernatant and add 300 µl bicarbonatebuffer + 1% DTAB (dodecyl(trimethyl)azanium bromide)
  - Mix thoroughly for cell lysis
  - Assay the neutralized samples using the NAD/NADH-Glo Assay by transferring 30 µl of each sample to the wells of a 96-well white luminometer plate and add 30µl of NAD/NADH-Glo Detection Reagent.
  - Incubate at room temperature and read luminescence after 30 to 60 minutes.
  - Determine NAD/NADH ratios by comparing RLU, or calculate the concentrations by comparison to a standard curve.
  - This method is used, if you want to measure the total amount of NAD^+ and NADH. For the determination of the individual NAD_+ and NADH concentrations follow the description below.
- Individual determination of NAD^+ and NADH
  - After the cells got mixed briefly transfer two 100µl aliquots to each of two new tubes. (R1 and R2)
  - Add 100µl HCl (0.4M) to R1 for acid treatment
  - Heat both aliquots at 60 °C for 15 minutes
  - Cool at room temperature for 10 minutes
  - Neutralize R1 with 100 µl trizma base (0.5M). This sample now contains the oxidized form NAD.
  - Neutralize R2 with 200µl of a 1:1 mixture of HCl (0.5M) and trizma base (0.5M). This sample now contains the oreduced form NADH
  - Both samples can be assayed as already described.This protocol is taken from "Bioluminescent Nicotinamide Adenine Dinucleotide Detection in Bacteria" of the Promega corporation. All measurements were carried out with the GloMax® Discover Multimode-Reader.